



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/600,201	06/20/2003	Vladimir I. Slepnev	19781/2042	5135
7590 EDWARDS ANGELL PALMER & DODGE LLP P O BOX 55874 BOSTON, MA 02205			EXAMINER BERTAGNA, ANGELA MARIE	
		ART UNIT 1637	PAPER NUMBER PAPER	
		MAIL DATE 07/09/2007	DELIVERY MODE PAPER	

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/600,201	SLEPNEV, VLADIMIR I.	
	Examiner	Art Unit	
	Angela Bertagna	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) Responsive to communication(s) filed on 26 April 2007.
- 2a) This action is FINAL. 2b) This action is non-final.
- 3) Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) Claim(s) 1-75 is/are pending in the application.
 - 4a) Of the above claim(s) 74 and 75 is/are withdrawn from consideration.
- 5) Claim(s) _____ is/are allowed.
- 6) Claim(s) 1-73 is/are rejected.
- 7) Claim(s) _____ is/are objected to.
- 8) Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) The specification is objected to by the Examiner.
- 10) The drawing(s) filed on _____ is/are: a) accepted or b) objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
 - a) All b) Some * c) None of:
 1. Certified copies of the priority documents have been received.
 2. Certified copies of the priority documents have been received in Application No. _____.
 3. Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).
- * See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|--|---|
| 1) <input checked="" type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413)
Paper No(s)/Mail Date. _____ |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | 5) <input type="checkbox"/> Notice of Informal Patent Application |
| 3) <input type="checkbox"/> Information Disclosure Statement(s) (PTO/SB/08)
Paper No(s)/Mail Date _____ | 6) <input type="checkbox"/> Other: _____ |

DETAILED ACTION

Status of the Application

1. Applicant's response filed on April 26, 2007 is acknowledged. Claims 1-75 are currently pending. Claims 74 and 75 are withdrawn from consideration as being drawn to a non-elected invention.

Claim Rejections - 35 USC § 102

2. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

3. Claims 1, 2, 5, 10, 12, 18-19, 22, 27, 34-35, 38 and 43 are rejected under 35 U.S.C. 102(b) as being anticipated by Myakishev et al. (Genome Research (Jan. 2001) 11: 163-169; cited previously).

Regarding claim 1, Myakishev teaches a method of determining for a given nucleic acid sample, the identity of the nucleotide at a known polymorphic site (see Figure 2 and Results section, pages 163-165), said method comprising:

a) subjecting to an amplification regimen a population of primer extension products generated from a nucleic acid sample (Figure 2, top panel, where extension with the tailed allele-specific primers occurs in round 1 of the amplification to generate the population of primer extension products; see also page 164, col. 2; steps 3-4 shown in Figure 2 and page 164, col. 2 teach amplification of these primer extension products),

each primer extension product comprising a tag sequence, which tag sequence specifically corresponds to the presence of one specific nucleotide at a known polymorphic site (page 164, column 1, teaches that the each allele-specific primer contains a different 21-base tail at the 5'end),

wherein said amplification regimen is performed using an upstream amplification primer (the "reverse primer" of page 163, col. 2 and page 164, col. 2) and a set of distinguishably labeled downstream amplification primers (the two energy-transfer (ET) primers of Fig. 2, see also page 164, col. 2), each member of said set of downstream amplification primers comprising a said tag sequence comprised by a member of said population of primer extension products and a distinguishable label (see Fig. 1 for the structure of the ET primers), wherein each distinguishable label specifically corresponds to the presence of a specific nucleotide at said polymorphic site (Fig. 2 teaches red and green labels for different mutations)

b) detecting incorporation of a distinguishable label into a nucleic acid molecule, thereby to determine the identity of the nucleotide at said polymorphic site (see Fig. 2 and pages 164-165).

Regarding claim 2, the distinguishable label is fluorescent (see Fig. 2 and page 164).

Regarding claim 5, the amplification reaction comprises at least two amplification reaction cycles, wherein each cycle comprises the steps of: (1) nucleic acid strand separation, (2) oligonucleotide primer annealing, and (3) polymerase extension of annealed primers (see Figure 2 and page 164; see also Methods section, page 167-168 for the reaction conditions).

Regarding claim 10, the tag sequence is 21 nucleotides (page 164, column 1).

Regarding claim 12, Myakishev teaches a pair of fluorescently-labeled downstream amplification primers each including a tag added in the first round of extension and corresponding to the presence of a first and second allele, respectively, at the polymorphic site (Figure 1 teaches the structure of the ET primers; see also page 164, col. 2).

Regarding claim 18, Myakishev teaches a method of determining, for a given nucleic acid sample, the identities of the nucleotides at a set of known polymorphic sites to be interrogated (see Fig. 2 and pages 163-165; note that 9 SNPs were tested), said method comprising:

a) subjecting to an amplification regimen, a population of primer extension products generated from a nucleic acid sample (Figure 2, top panel, where extension with the tailed primers occurs in round 1 of the amplification to generate the population of primer extension products; also page 164, col. 2; steps 3-4 shown in Figure 2 and page 164, col. 2 teach amplification of these primer extension products),

each primer extension product comprising a member of a set of tag sequences, which tag sequence specifically corresponds to the presence of one specific nucleotide at a known polymorphic site (page 164, col. 1 teaches that the allele-specific primers contain different 5' tags),

wherein said amplification regimen is performed using one upstream amplification primer for each sequence comprising a known polymorphic site to be interrogated (the reverse primer, page 163), and a set of distinguishably labeled downstream amplification primers (the ET primers; Fig. 2),

each member of said set of downstream amplification primers comprising a said tag sequence comprised by a member of said population of primer extension products (see Figure 1) and a distinguishable label that specifically corresponds to the presence of a specific nucleotide at said polymorphic site (see Figures 1 & 2), and wherein said upstream amplification primers are selected such that each polymorphic site of said set of known polymorphic sites to be interrogated corresponds to a distinctly sized amplification product (see page 164, col. 1, where the upstream primer was hybridized at different points on the template to produce differently sized amplicons)

b) detecting incorporation of a distinguishable label in distinctly sized amplification products, thereby to determine the identity of the nucleotide at each said polymorphic site (see Fig. 2 and also the Methods section, page 168, where fluorescence is detected).

Regarding claim 19, the label is fluorescent (see Fig. 2).

Regarding claim 22, the amplification reaction comprises at least two amplification reaction cycles, wherein each cycle comprises the steps of: 1) nucleic acid strand separation; 2) oligonucleotide primer annealing; and 3) polymerase extension of annealed primers (see Figure 2 and page 164; see also Methods section, page 167-168 for the reaction conditions).

Regarding claim 27, Myakishev teaches that the tag sequence is 21 nucleotides (page 164, col. 1).

Regarding claim 34, Myakishev teaches a method of determining, for a given nucleic acid sample, the identities of the nucleotides at a set of known polymorphic sites to be interrogated (see Fig. 2 and pages 163-165; note that 9 SNPs were tested), said method comprising:

Art Unit: 1637

a) subjecting to an amplification regimen, a population of primer extension products generated from a nucleic acid sample (Figure 2 and page 164, col. 2), each primer extension product comprising a first tag sequence (generated by extension of the reverse primer) or its complement and a member of a set of second tag sequences or its complement (the 21 nt tail added in step 1 of the reaction), the presence of which second tag sequence or its complement specifically corresponds to the presence of one specific nucleotide at a known polymorphic site (page 164, col. 1), wherein for each polymorphic site in said set of polymorphic sites, said first tag sequence is located at a distinct distance 5' of said polymorphic site, relative to the distance of said first tag sequence from a polymorphic site on molecules in said sample containing other polymorphic sites (page 164, col. 1 teaches that the reverse primer may hybridize at different positions on the template), wherein said amplification regimen is performed using an upstream amplification primer comprising said first tag sequence (the reverse primer, page 163), and a set of distinguishably labeled downstream amplification primers (the ET primers), each member of said set of downstream amplification primers comprising a said tag sequence comprised by a member of said population of primer extension products (the tail sequence; see Fig. 1) and a distinguishable label that specifically corresponds to the presence of a specific nucleotide at said polymorphic site (see Fig. 1 and 2), and wherein said upstream amplification primers are selected such that each polymorphic site of said set of known polymorphic sites to be interrogated corresponds to a distinctly sized amplification product (page 164, col. 1 teaches hybridization of the reverse primer at different locations on the template)

Art Unit: 1637

b) detecting incorporation of a distinguishable label in distinctly sized amplification products, thereby to determine the identity of the nucleotide at each said polymorphic site (page 168, col. 1).

Regarding claim 35, Myakishev teaches fluorescent labels (Fig. 2).

Regarding claim 38, the amplification reaction comprises at least two amplification reaction cycles, wherein each cycle comprises the steps of: 1) nucleic acid strand separation; 2) oligonucleotide primer annealing; and 3) polymerase extension of annealed primers (see Figure 2 and page 164; see also Methods section, page 167-168 for the reaction conditions).

Regarding claim 43, the tag is 21 nucleotides in length (page 164, col. 1).

Claim Rejections - 35 USC § 103

4. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

5. Claims 3, 4, 9, 20, 21, 26, 36, 37, and 42 are rejected under 35 U.S.C. 103(a) as being unpatentable over Myakishev et al. (Genome Research (Jan. 2001) 11: 163-169; cited previously) in view of Piggee et al. (J Chromatogr A. 1997 Sep 26; 781(1-2): 367-75; cited previously).

Myakishev teaches the method of claims 1, 18, and 34, as discussed above.

Myakishev teaches fluorescence detection of amplified products rather than capillary electrophoresis separation and detection.

Regarding claims 9, 26, and 42, Myakishev teaches a thermal cycling device with a fluorescence detection system (Methods, page 168). Note that the specification does not require that the elements of the modular apparatus of claims 9, 26, and 42 (a thermal cycling device, a sampling device, a capillary electrophoresis device and a fluorescence detector) be physically connected.

Regarding claims 3-4, 20-21, 36, and 37, Piggee teaches a method of detecting point mutations using capillary electrophoresis and laser-induced fluorescence detection (see abstract).

Regarding claims 9, 26, and 42, Piggee teaches a capillary electrophoresis device with a sampling device, and a fluorescence detection system (pages 368-369).

Piggee teaches that this method is fast, avoids radioactive labels, and has a high automation potential. Although Piggee used capillary electrophoresis to detect point mutations analyzed by single base extension reactions, it is expressly stated that the method would also be applicable to other methods of mutation detection including competitive priming with end mismatch, etc. Piggee also noted that the capillary electrophoresis method facilitated multiplexing through the use of different length primers (see Conclusion section, page 375).

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to utilize capillary electrophoresis as an additional or alternative method of detection in the mutation detection method of Myakishev. Piggee expressly taught the advantages of a capillary electrophoresis detection system, namely, rapid analysis with a high potential for automation and multiplexing (see above). The ordinary practitioner would have been motivated by the teachings of Piggee to additionally (or alternatively) analyze the SNP detection results using capillary electrophoresis in order to more rapidly and automatically screen a larger number

of samples with the additional control of length-based detection, thus resulting in the instantly claimed methods.

6. Claims 6-8, 23-25, and 39-41 are rejected under 35 U.S.C. 103(a) as being unpatentable over Myakishev et al. (Genome Research (Jan. 2001) 11: 163-169; cited previously) in view of Wiesner et al. (Biochemical and Biophysical Research Communications (1992) 183(2): 553-559; cited previously) and further in view of Piggee et al. (J Chromatogr A. 1997 Sep 26; 781(1-2): 367-75; cited previously).

Myakishev teaches the method of claims 1-2, 5, 10, 12, 18-19, 22, 27, 34-35, 38 and 43, as discussed above.

Myakishev does not teach analyzing an aliquot of the PCR during the reaction process. Also, Myakishev does not teach capillary electrophoresis.

Wiesner teaches a method of determining the initial concentration of a PCR template by measuring the amount of product accumulating in consecutive cycles (see abstract).

Regarding claims 6-7, 23-24, 39, and 40, Wiesner teaches taking an aliquot after each cycle of a PCR reaction and analyzing the amount of accumulating product by agarose gel electrophoresis and scintillation counting (page 554, Methods section). Wiesner teaches that by analyzing aliquots the need for standardization of the signal due to differing initial product concentrations is eliminated. The teachings of Wiesner also permit optimization of cycling parameters and an early identification of the reaction product (page 555, where the product is detectable halfway through the amplification reaction).

Wiesner does not teach analysis using capillary electrophoresis.

Regarding claims 8, 25, and 41, Piggee teaches a method of detecting point mutations using capillary electrophoresis and laser-induced fluorescence detection (see abstract).

Piggee teaches that this method is fast, avoids radioactive labels, and has a high automation potential. Although Piggee used capillary electrophoresis to detect point mutations analyzed by single base extension reactions, it is expressly stated that the method would also be applicable to other methods of mutation detection including competitive priming with end mismatch, etc. Piggee also noted that the capillary electrophoresis method facilitated multiplexing through the use of different length primers (see Conclusion section, page 375).

It would have been *prima facie* obvious for one of ordinary skill in the art at the time of invention to incorporate the teachings of Wiesner and Piggee into the genotyping method of Myakishev. Wiesner particularly pointed out the advantages of taking an aliquot after each cycle of the amplification reaction, namely: the ability to rapidly optimize the reaction conditions, to quickly determine the identity and quantity of the product, and also determine the initial concentration of the template for standardization purposes (see above). The ordinary practitioner would have been motivated by the teachings of Wiesner to analyze an aliquot of the PCR reaction of Myakishev in order to incorporate the above advantages into the method. Furthermore, the ordinary practitioner would have been motivated by the teachings of Piggee to substitute capillary electrophoresis for the agarose electrophoresis and scintillation counting taught by Wiesner. As discussed above, Piggee taught that capillary electrophoresis offered a rapid, radiation-free, automation-friendly, and high-throughput method for analyzing DNA samples (see above). The ordinary artisan, interested in combining the teachings of Myakishev and Wiesner in order to more rapidly detect mutations, would have been further motivated to use

Art Unit: 1637

capillary electrophoresis, as suggested by Piggee, in order to further increase the speed, multiplexing ability, automation and throughput of the assay. Therefore, the combined teachings of Myakishev, Wiesner, and Piggee result in the instant claims 6-8, 23-25, and 39-41.

7. Claims 11, 13-17, 28-33, 44-52, 54, 58-64, 66, and 70-73 are rejected under 35 U.S.C. 103(a) as being unpatentable over Myakishev et al. (Genome Research (Jan. 2001) 11: 163-169; cited previously) in view of Nolan et al. (USPN 6,287,766 B1; cited previously).

Myakishev teaches the method of claims 1-2, 5, 10, 12, 18-19, 22, 27, 34-35, 38 and 43, as discussed above.

Regarding claims 50 and 62, Myakishev teaches a method of determining the identity of a single nucleotide at a known polymorphic site (see Fig. 2, and pages 163-165), said method comprising:

- I) providing a nucleic acid sample comprising said polymorphic site (Fig. 2)
- II) separating the strands of said nucleic acid sample and re-annealing in the presence of:
 - a) a first oligonucleotide primer comprising a 3' region that hybridizes to a sequence at a known distance downstream of said known polymorphic site, said first oligonucleotide primer comprising a first sequence tag located 5' of said 3' region (the reverse primer of Fig. 2; note that since the specification defines a tag as any nucleic acid sequence in a primer, any dinucleotide of the reverse primer is a tag)
 - b) a set of second oligonucleotide primers (the allele-specific primers of Fig. 2), wherein each member of said set comprises:
 - i) a region that hybridizes 5' of and adjacent to said polymorphic site

- ii) a variable 3' terminal nucleotide, wherein, when said member is hybridized to said known sequence, said 3' terminal nucleotide is opposite said polymorphic site, and wherein, if and only if said 3' terminal nucleotide is complementary to the nucleotide at said polymorphic site, said 3' terminal nucleotide base pairs with said nucleotide at said polymorphic site (see Fig. 2 and page 164)
 - iii) a tag sequence that corresponds to said variable 3'-terminal nucleotide of (ii), said tag sequence located 5' of the region of (i) on said member (see the tail sequence in Fig. 2)
- III) contacting the annealed oligonucleotides resulting from step (II) with a nucleic acid polymerase under conditions that permit the extension of an annealed oligonucleotide such that extension products are generated, wherein the primer extension product from the first oligonucleotide primer, when separated from its complement, can serve as a template for the synthesis of the extension product of a member of the set of second oligonucleotide primers (see Fig. 2), and vice versa
- IV) repeating strand separating and contacting steps (II) and (III) two times, such that a population of nucleic acid molecules is generated that comprises both a sequence identical to or complementary to said first oligonucleotide and a sequence identical to or complementary to one of the members of said second set of oligonucleotides (see Fig. 2 and page 164)

V) subjecting said population of nucleic acid molecules to an amplification regimen, wherein said amplification regimen is performed using an upstream amplification primer comprising the first sequence tag comprised by said first oligonucleotide primer (the reverse primer, as discussed above), and a set of downstream amplification primers, each member of said

Art Unit: 1637

set of downstream amplification primers comprising a tag comprised by a member of said set of second oligonucleotide primers and a distinguishable label (the energy transfer primers that comprise the tail sequence; see Fig. 1)

VIII) detecting incorporation of at least one distinguishable label, thereby determining the identity of the nucleotide at said known polymorphic site (see Fig. 2 and page 168).

Note that the designations “upstream” and “downstream” are arbitrary designations depending on the choice of the reference strand, and therefore, the primers of Myakishev meet the claimed limitations.

Regarding claims 51 and 63, Myakishev teaches fluorescent labels (Fig. 2).

Regarding claims 54 and 66, the amplification reaction comprises at least two amplification reaction cycles, wherein each cycle comprises the steps of: 1) nucleic acid strand separation; 2) oligonucleotide primer annealing; and 3) polymerase extension of annealed primers (see Figure 2 and page 164; see also Methods section, page 167-168 for the reaction conditions).

Regarding claims 58 and 70, Myakishev teaches that the tail portions of the allele-specific primers are 21 nucleotides in length (page 164, col. 1). Also, since the entire 20 nt reverse primer comprises a tag, both the first and second tags comprise 15 to 40 nucleotides.

Regarding claims 59, 60, 71, and 72, the 20 nt reverse primer hybridizes with perfect complementarity to the target downstream of the polymorphic site, and the allele-specific primers have a 22 nt target-specific portion that hybridizes upstream of the polymorphic site (see Table 1).

Myakishev does not teach exonuclease digestion to remove unextended primers as required by claims 13-17, 29-33, 45-50, and 62. Also, regarding claim 62, Myakishev does not teach the use of multiple first primers.

Regarding claims 52 and 64, Myakishev does not teach separating the nucleic acid molecules by size and/or charge.

Regarding claims 11, 28, 44, 61 and 73, Myakishev teaches using a set of downstream primers comprising two different nucleotide bases at the 3'end, for example, A and G (see Figure 2), but does not teach using a set of downstream primers consisting of four primers, each with a different 3'-terminal nucleotide.

Nolan teaches a method of identifying polymorphisms using flow cytometry.

Regarding claims 13-17, 29-33, 45-50, and 62, Nolan teaches an embodiment wherein unincorporated primers from an initial amplification reaction may be degraded using the heat labile Exonuclease I followed by polymerase extension, where the initial denaturation step destroys the activity of the exonuclease (see for example, column 5, line 60 – column 6, line 20). Nolan also teaches multiplex detection of mutations using multiple sets of primers (column 7, lines 60-63).

Regarding claims 52 and 64, Nolan teaches separation of the amplified products by flow cytometry (column 7, lines 60-63).

Regarding claims 11, 28, 44, 61 and 73, the method taught by Nolan in Example 5 (column 7, lines 1-63) comprises an oligonucleotide ligation assay (OLA) followed by PCR amplification with an upstream primer and a set of downstream primers that may be fluorescently labeled (column 7, lines 11-50). Nolan teaches that the downstream primers each

have a different 3'terminal nucleotide (column 7, lines 27-30) in order to identify the polymorphism in a single reaction.

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to incorporate the teachings of Nolan into the SNP genotyping method of Myakishev. An ordinary practitioner would have recognized that not all polymorphic sites may be genotyped using only two allele-specific downstream primers, and therefore, would have been motivated by the teachings of Nolan to utilize a subset of four downstream primers each with a different 3'terminus, in order to accurately type these polymorphisms. Also, an ordinary practitioner would have been motivated by the teachings of Nolan to perform a multiplexed analysis, utilizing multiple primer pairs in order to more rapidly and reproducibly genotype several sites simultaneously. Furthermore, the person of ordinary skill would have been motivated by Nolan to incorporate an exonuclease digestion step following the initial primer extension reaction with the allele-specific primers. Although Myakishev attempted to avoid mispriming by careful design of the allele-specific and energy-transfer primers (see page 164, col. 1), incorporation of the exonuclease digestion step taught by Nolan following the initial extension would have eliminated the possibility of such mispriming events, and thereby improved the accuracy of the method. Therefore, one of ordinary skill in the art, interested in obtaining a method to more rapidly and accurately determine the identity of multiple polymorphic sites using the method of Myakishev, would have been motivated to use multiple primer sets where the downstream primer sets contain all possible 3'terminal nucleotides and also to incorporate an exonuclease digestion step as suggested by Nolan, thus resulting in the instantly claimed methods.

8. Claims 53, 57, 65, and 69 are rejected under 35 U.S.C. 103(a) as being unpatentable over Myakishev et al. (Genome Research (Jan. 2001) 11: 163-169; cited previously) in view of Nolan et al. (USPN 6,287,766 B1; cited previously) and further in view of Piggee et al. (J Chromatogr A. 1997 Sep 26; 781(1-2): 367-75; cited previously).

The combined teachings of Myakishev and Nolan result in the instant claims 50, 62, and 64, as discussed above.

Regarding claims 57 and 69, Myakishev teaches a thermal cycling device with a fluorescence detection system (Methods, page 168). Note that the specification does not require that the elements of claims 57 and 69 (a thermal cycling device, a sampling device, a capillary electrophoresis device and a fluorescence detector) be physically connected.

Neither Myakishev nor Nolan teaches the use of capillary electrophoresis.

Regarding claims 53 and 65, Piggee teaches a method of detecting point mutations using capillary electrophoresis and laser-induced fluorescence detection (see abstract).

Regarding claims 57 and 69, Piggee teaches a capillary electrophoresis device with a sampling device, and a fluorescence detection system (pages 368-369).

Piggee teaches that this method is fast, avoids radioactive labels, and has a high automation potential. Although Piggee used capillary electrophoresis to detect point mutations analyzed by single base extension reactions, it is expressly stated that the method would also be applicable to other methods of mutation detection including competitive priming with end mismatch, etc. Piggee also noted that the capillary electrophoresis method facilitated multiplexing through the use of different length primers (see Conclusion section, page 375).

Art Unit: 1637

It would have been *prima facie* obvious for one of ordinary skill in the art at the time of invention to utilize capillary electrophoresis as an additional or alternative method of detection in the mutation detection method resulting from the combined teachings of Myakishev and Nolan. Piggee expressly taught the advantages of a capillary electrophoresis detection system, namely, rapid analysis with a high potential for automation and multiplexing (see above). An ordinary practitioner would have been motivated by the teachings of Piggee to additionally (or alternatively) analyze the SNP detection results using capillary electrophoresis in order to more rapidly and automatically screen a larger number of samples, thus resulting in the instantly claimed methods.

9. Claims 55-56, 67, and 68 are rejected under 35 U.S.C. 103(a) as being unpatentable over Myakishev et al. (*Genome Research* (Jan. 2001) 11: 163-169; cited previously) in view of Nolan et al. (USPN 6,287,766 B1; cited previously) and further in view of Wiesner et al. (*Biochemical and Biophysical Research Communications* (1992) 183(2): 553-559; cited previously).

The combined teachings of Myakishev and Nolan result in the instant claims 54 and 66, as discussed above.

Neither Myakishev nor Nolan teaches analyzing an aliquot of the PCR during the reaction process.

Wiesner teaches a method of determining the initial concentration of a PCR template by measuring the amount of product accumulating in consecutive cycles (see abstract).

Regarding claims 55, 56, 67, and 68, Wiesner teaches taking an aliquot after each cycle of a PCR reaction and analyzing the amount of accumulating product by agarose gel

electrophoresis and scintillation counting (page 554, Methods section). Wiesner teaches that by analyzing aliquots the need for standardization of the signal due to differing initial product concentrations is omitted. The teachings of Wiesner also permit optimization of cycling parameters and an early identification of the reaction product (page 555, where the product is detectable halfway through the amplification reaction).

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to incorporate the teachings of Wiesner into the genotyping method resulting from the combined teachings of Myakishev and Nolan. Wiesner particularly pointed out the advantages of taking an aliquot after each cycle of the amplification reaction, namely: the ability to rapidly optimize the reaction conditions, to quickly determine the identity and quantity of the product, and also determine the initial concentration of the template for standardization purposes (discussed above). The ordinary practitioner would have been motivated by the teachings of Wiesner to analyze an aliquot of the PCR reaction generated in the method of Myakishev and Nolan in order to incorporate the above advantages into the method. Therefore, the ordinary artisan, interested in more rapidly detecting mutations, would have been motivated to take an aliquot of the amplification reaction for analysis, as suggested by Wiesner, thus resulting in the methods of the instant claims 55, 56, 67, and 68.

Response to Arguments

10. Regarding the rejection of claims 1, 2, 5, 10, 12, 18-19, 22, 27, 34-35, 38 and 43 under 35 U.S.C. 102(b) as being anticipated by Myakishev, Applicant's arguments filed April 26, 2007 have been fully considered, but they are not persuasive. Applicant argues that Myakishev does

Art Unit: 1637

not teach all of the limitations of the claims, specifically the requirement that the amplification step is conducted using an upstream primer and a set of distinguishably labeled downstream primers (see page 16 of the response). This argument was not found persuasive, because as noted above and also in the instant application's specification (see page 19), the designations "upstream primer" and "downstream primer" are arbitrary designations depending on the choice of the reference strand. In other words, the same primer may be designated an "upstream primer" or a "downstream primer" depending on the choice of the reference strand. Likewise, the "forward" and "reverse" designations used by Myakishev to describe the primers shown in Figure 2 are arbitrary designations, and therefore, the set of energy-transfer primers used in steps 3-4 of the method (see Figure 2) may be considered "upstream primers" or "downstream primers" depending on the choice of the reference strand. Furthermore, when the method of Myakishev is applied to detection of the DRD2 SNP, for example, the energy transfer primers hybridize downstream of the polymorphic base in three of the six possible reading frames for this sequence (see below, where the GenBank sequence of DRD2 recited in Table 1 of Myakishev was translated using all six possible reading frames). In reading frames 4-6 below, the energy transfer primers of Myakishev hybridize downstream (3') of the polymorphic base. Therefore, Myakishev teaches the use of a set of distinguishably labeled downstream primers as required by the instant claims, and the rejection under 35 U.S.C. 102(b) is maintained.

Art Unit: 1637

Translation of GenBank Accession No. X51645 (shown in Table 1 of Myakishev) using six different reading frames:

(1) 5'-3', Frame 1

L AT Q S V H R P D G S T E S V L V **Stop Stop Stop Stop S** G E A E L E P A L Q R V R R E G G
 Q T P L Q L L C H T A H P A H R C H R L R Q R A G V H G C V P R E G A A D H H Q L P
 D R Q P R S G R P P R R H T G H A L G C L P G G G R **Stop V E I Q Q D S L Stop H L R H**
 S G R H D V H G E H P E L V C H Q H R Q V H S C G H A H A V Q Y A L Q L Q A P G H
 R H D L H R L G P V L H H L L P T P L R T Q **Stop R R P E R V H H C Q P G L R G L L L**
 H R L L L R A L H C H P A G L Y Q D L H C P P Q T P Q A S Q H Q T Q Q P S F Q G P P E
 G S T K G Q L Y S P R G H E T L H R Y H E V **Stop W E F P S E Q A E S G G C P A S P G**
 A G D G D A L Q H Q P T R E D P V Q P H P T Q P P P A D S P R P V P P W S P Q H S R Q
 P R Q T R E E W A C Q R P P Q D C Q D L **Stop D P D H A Q W Q N P D L P Q D H E P**
Stop E A L P A E G E E S H S D A R H C S R R V H H L L A A L L H H T H P E H T L Stop
 L Q H P A C P V Q R L H V A G L C Q Q R R E P H H L H H L Q H **Stop V P Q G L P E D P**
 P L L T L L P A R T A A C F P P P C P G R P A S P L R T V S R K A W V D R P P L H P G R
 P C S V R L A P C S S L P A

(2) 5'-3', Frame 2

W P P S R S T A L **Met D** P L N L S W Y D D D L E R Q N W S R P F N G S D G K A D R P
 H Y N Y Y A T L L T L L I A V I V F G N V L V C **Met A** V S R E K A L Q T T N Y L I V
 S L A V A D L L V A T L V **Met P** W V V Y L E V V G E W K F S R I H C D I F V T L D V
Met Met C T A S I L N L C A I S I D R Y T A V A **Met P Met L** Y N T R Y S S K R R V T V
Met I S I V W V L S F T I S C P L L F G L N N A D Q N E C I I A N P A F V V Y S S I V S F
 Y V P F I V T L L V Y I K I Y I V L R R R R K R V N T K R S S R A F R A H L R A P L K G
 N C T H P E D **Met K L C T V I Met K S N G S F P V N R R R V E A A R R A Q E L E Met**
E Met L S S T S P P E R T R Y S P I P P S H H Q L T L P D P S H H G L H S T P D S P A K P
E K N G H A K D H P K I A K I F E I Q T Met P N G K T R T S L K T Met S R R K L S Q Q
K E K K A T Q Met L A I V L G V F I I C W L P F F I T H I L N I H C D C N I P P V L Y S A
F T W L G Y V N S A V N P I I Y T T F N I E F R K A F L K I L H C Stop L C C L P A Q Q P
A S H L P A Q A G Q P H P C E P Stop A G R P G W I G L L F T P A G P A V F A W L H A
P H C P H

(3) 5'-3', Frame 3

G H P V G P P P **Stop W I H Stop I C P G Met Met Met I W R G R T G A G P S T G Q T G R**
 R T D P T T T T **Met P H C S P C S S L S S S A T C W C A W L C P A R R R C R P P P T T**
Stop S S A S Q W P T S S S P H W S C P G L S T W R W Stop V S G N S A G F T V T S S S
 L W T S **Stop C A R R A S S Stop T C V P S A S T G T Q L W P C P C C T I R A T A P S A G**
 S P S **Stop S P S S G S C P S P S P A H S S S D S I T Q T R T S A S L P T R P S W S T P P S**
S P S T C P S L S P C W S I S R S T L S S A D A A S E S T P N A A A E L S G P T Stop G L
H Stop R A T V L T P R T Stop N S A P L S Stop S L Met G V S Q Stop T G G E W R L P G
E P R S W R W R C S P A P A H P R G P G T A P S H P A T T S Stop L S P T R P T Met V S
T A L P T A P P N Q R R Met G Met P K T T P R L P R S L R S R P C P Met A K P G P P S

Art Unit: 1637

R P Stop A V G S S P S R R R R K P L R C S P L F S A C S S A G C P S S S H T S Stop T
 Y T V T A T S R L S C T A P S R G W A Met S T A P Stop T P S S T P P S T L S S A R P S
 Stop R S S T A D S A A C P H S S L L P T S L P R P A S L T L A N R E Q E G L G G S A S
 S S P R Q A L Q C S L G S Met L L T A R

(4) 3'-5', Frame 1

V R A V R S Met E P S E H C R A C R G E E E A D P P R P S C S R F A R V R L A G L G R
 E V G S R L L C G Q A A E S A V E D L Q E G L A E L N V E G G V D D G V H G A V D I
 A Q P R E G A V Q D R R D V A V T V Y V Q D V C D E E G Q P A D D E H A E N N G E
 H L S G F L L L L G E L P T A H G L E G G P G F A I G H G L D L K D L G N L G V V F
 G Met P I L L W F G G A V G S A V E T Met V G R V G E S Q L V V A G W D G A V P G P
 L G W A G A G E H L H L Q L L G S P G S L H S P P V H W E T P I R L H D N G A E F H V
 L G V S T V A L Stop W S P Q V G P E S S A A A F G V D S L A A S A E D N V D L D I D
 Q Q G D N E G H V E G D D G G V D H E G R V G N D A L V L V C V I E S E E E W A G
 D G E G Q D P D D G D H D G D P A L G A V A R I V Q H G H G H S C V P V D A D G T
 Q V Q D A R R A H H D V Q S D E D V T V N P A E F P L T Y H L Q V D N P G H D Q C G
 D E E V G H C E A D D Q V V G G G L Q R L L A G H S H A H Q H V A E D D D S D E Q
 G E Q C G I V V V V V G S V R L P V Stop P V E G P A P V L P L Q I I I P G Q I Q W I H Q
 G G G P T G W P

(5) 3'-5', Frame 2

C G Q Stop G A W S Q A N T A G P A G V K R R P I H P G L P A H G S Q G Stop G W P A
 W A G R W E A G C C A G R Q Q S Q Q W R I F R K A L R N S Met L K V V Stop Met Met
 G F T A L L T Stop P S H V K A L Y R T G G Met L Q S Q C Met F R Met C V Met K K G S
 Q Q Met Met N T P R T Met A S I Stop V A F F S F C W E S F L R L Met V L R E V R V L
 P L G Met V W I S K I L A I L G W S L A C P F F S G L A G L S G V L W R P W W D G S G
 R V S W W W L G G Met G L Y R V L S G G L V L E S I S I S S W A R R A A S T L R L F
 T G K L P L D F Met I T V Q S F Met S S G Stop V Q L P F S G A L R W A L K A R L L R L
 V L T R L R R L R T Met Stop I L I Stop T S R V T Met K G T Stop K E T Met E E Stop T
 T K A G L A Met Met H S F W S A L L S P K R S G Q E Met V K D R T Q T Met E I Met T
 V T R R L E L Stop R V L Y S Met G Met A T A V Y L S Met L Met A H K F R Met L A V
 H I Met T S R V T K Met S Q Stop I L L N F H S P T T S R Stop T T Q G Met T S V A T R R
 S A T A R L T I R Stop L V V V C S A F S R D T A Met H T S T L P K T Met T A Met S R V
 S S V A Stop Stop L Stop W G L S A F P S D P L K G R L Q F C L S R S S S Y Q D R F S G
 S I R A V D R L G G Q

(6) 3'-5', Frame 3

A G S E E H G A K R T L Q G L P G Stop R G G R S T Q A F L L T V R K G E A G R P G Q
 G G G K Q A A V R A G S R V V S S G G S S G R P C G T Q C Stop R W C R Stop W G S R R
 C Stop H S P A T Stop R R C T G Q A G C C S H S V C S G C V Stop Stop R R A A S R
 Stop Stop T R R E Q W R A S E W L S S P S A G R A S Y G S W S Stop G R S G F C H W A
 W S G S Q R S W Q S W G G L W H A H S S L V W R G C R E C C G D H G G T G R G E S
 A G G G W V G W G C T G S S R V G W C W R A S P S P A P G L A G Q P P L S A C S L G
 N S H Stop T S Stop Stop R C R V S C P R G E Y S C P L V E P S G G P Stop K L G C C V

Art Unit: 1637

W C Stop L A C G V C G G Q C R S Stop Y R P A G Stop Q Stop R A R R R R R W R S R P
R R P G W Q Stop C T R S G L R Y Stop V R R G V G R R W Stop R T G P R R W R S Stop
R Stop P G A W S C S A Y C T A W A W P Q L C T C R C Stop W H T S S G C S P C T S
Stop R P E Stop R R C H S E S C Stop I S T H L P P P G R Q P R A Stop P V W R R G G R P
L R G Stop R S G S W W W S A A P S R G T Q P C T P A R C R R R Stop Q R Stop A G
Stop A V W H S S C S G V C P P S R L T R Stop R A G S S S A S P D H H H T R T D S V D
P S G R W T D W V A

Applicant's arguments regarding the rejection of claims 3, 4, 6-9, 11, 13-17, 20, 21, 23-26, 28-33, 36, 37, 39-42, and 44-73 under 35 U.S.C. 103(a) were also not found persuasive. Regarding the rejection of claims 3, 4, 9, 20, 21, 26, 36, 37, and 42 under 35 U.S.C. 103(a) as being unpatentable over Myakishev in view of Piggee, Applicant first argues that the cited references do not teach all of the claimed limitations, specifically the distinguishably labeled downstream primers (page 17). This argument was not found persuasive, because as discussed above, Myakishev teaches all of the elements of claims 1, and Piggee is only relied upon for those teachings relevant to the dependent claims. Applicant also argues that the teachings of Myakishev and Piggee cannot be combined, because the method of Myakishev requires primer extension and PCR amplification, whereas the method taught by Piggee comprises single base extension using terminator nucleotides (pages 17-18). This argument was not found persuasive, because the teachings of Piggee regarding the advantages of capillary electrophoresis-based detection methods would be highly relevant to the ordinary practitioner of the method taught by Myakishev. As noted above, an ordinary practitioner would have been motivated to use capillary electrophoresis detection in order to obtain a rapid, simple, high-throughput, and readily automated detection method. Furthermore, the test for obviousness is not whether the features of a secondary reference may be bodily incorporated into the structure of the primary reference; nor is it that the claimed invention must be expressly suggested in any one or all of the references.

Art Unit: 1637

Rather, the test is what the combined teachings of the references would have suggested to those of ordinary skill in the art. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981).

Regarding the rejection of claims 6-8, 11, 13-17, 23-25, 28-33, 39-41, and 44-73 under 35 U.S.C. 103(a), Applicant argues that the cited references do not teach all of the claimed limitations, specifically, the set of distinguishably labeled downstream primers (see pages 19-21). This argument was not found persuasive, because as discussed above, Myakishev teaches the set of distinguishably labeled downstream primers. The secondary references are only relied upon for those teachings relevant to the dependent claims. Accordingly, the rejections are maintained.

Conclusion

11. No claims are currently allowable.

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. Hawkins et al. (Human Mutation (2002) teaches allele-specific PCR using tailed forward or tailed reverse primers, where the tail specifically identifies the polymorphic base (see Table 1 and Figure 1). Woolley et al. (Analytical Chemistry (1996) 68(23): 4081-4086) teaches an integrated DNA analysis system comprising a thermal cycler and capillary electrophoresis system (see abstract and Figure 1).

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO

Art Unit: 1637

MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Angela Bertagna whose telephone number is (571) 272-8291. The examiner can normally be reached on M-F 7:30-5 pm EST.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Angela Bertagna
Art Unit 1637 *AMB*
June 27, 2007

amb


JEFFREY FREDMAN
PRIMARY EXAMINER

6/28/07